AFRRI SR74-15
JULY 1974

AFRRI
SCIENTIFIC REPORT

MAMMALIAN COLD RECEPTOR AFFERENTS: ROLE OF AN ELECTROGENIC SODIUM PUMP IN SENSORY TRANSDUCTION

D. O. Carpenter
Fr.-K. Pierau
P. J. Torrey

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

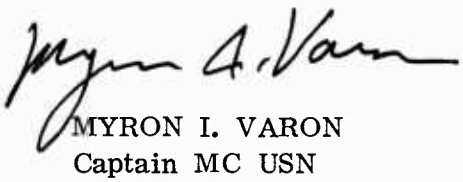
Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.

AFRRI SR74-15
July 1974

MAMMALIAN COLD RECEPTOR AFFERENTS: ROLE OF AN ELECTROGENIC SODIUM PUMP IN SENSORY TRANSDUCTION

D. O. CARPENTER
FR.-K. PIERAU
P. J. TORREY

D. O. CARPENTER
Chairman
Neurobiology Department



MYRON I. VARON
Captain MC USN
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

TABLE OF CONTENTS

LIST OF FIGURES

ABSTRACT

The responses of specific cold-sensitive afferents and cold-sensitive mechanoreceptors were recorded from rat pudendal nerve. A local infiltration of ouabain into the region of the afferent terminals caused a dramatic increase in discharge at warm temperatures without significant effect on discharge in the cold. These results suggest that an electrogenic sodium pump is the generator potential mechanism imparting cold sensitivity.

I. INTRODUCTION

The afferent discharge characteristic of specific peripheral cold receptors responding to temperature changes is well known from experiments in a variety of mammals and man.[4,5,7] However, the transduction process which underlies this temperature-dependent discharge is unknown. Since specific thermoreceptors are small in diameter and difficult to study, mechanoreceptors have been studied as models of thermoreceptors. Some mechanoreceptors have a fairly high temperature sensitivity. In Pacinian corpuscles the generator potential increases with rising temperature and consequently there is a greater discharge rate at higher temperatures.[10] However, the discharge frequency of cat muscle spindle afferents increases with cooling,[9] supposedly due to a depolarizing effect of the fall in temperature at the terminals. But none of these experiments provided an understanding of the generator potential mechanism in specific or even nonspecific temperature sensitive receptors.

Recent studies of neurons which are not primary temperature receptors have demonstrated mechanisms which might explain the temperature sensitivity of specific thermoreceptors. In Aplysia neurons, which have a very high specific membrane resistance, a ouabain sensitive electrogenic sodium pump contributes to membrane potential and tends to cause an increase in excitability on cooling, as a result of the high temperature dependence of this active transport process.[2,3] This depolarizing effect of cooling is opposed by a greater temperature coefficient of the permeability of sodium (P_{Na^+}) than potassium (P_{K^+}), which tends to hyperpolarize and consequently to decrease the excitability at lower temperatures.

However, in mammalian spinal motor neurons[8] a greater temperature coefficient of P_{K^+} than P_{Na^+} leads to a depolarization during cooling which is accompanied by an increase in membrane resistance. As a result these cells are more excitable at cold temperatures. Thus, these studies suggest two possible mechanisms for cold transduction, and one for warm transduction.

We have attempted to evaluate whether an electrogenic sodium pump is a mechanism determining the excitability of mammalian cold-sensitive primary afferent fibers by a local application of ouabain into the region of the afferent terminal. Ouabain is an inhibitor of sodium-potassium adenosine triphosphatase (Na^+-K^+-ATPase), the enzyme thought to be responsible for the operation of the sodium transport process,[1] and is a relatively specific blocking drug for electrogenic pumps in other preparations.[2,3]

II. METHODS

The effects of local ouabain infiltration were tested on temperature-dependent activity of afferent fibers isolated from the pudendal nerve of rats. This nerve contains a large number of fibers conducting action potentials from specific cold and warm receptors and from temperature sensitive mechanoreceptors in the scrotal skin.[6] The temperature of the scrotal skin was controlled using a metal thermode containing circulating water at different temperatures. A small thermocouple at the surface of the thermode measured temperature between the thermode and the scrotal skin. Small nerve filaments were isolated from the pudendal nerve of rats anesthetized with Equi-Thesin.* The afferent spike potentials were recorded in a

* Each 100 ml contains 5.24 g chloral hydrate, 1.0 g pentobarbital, 2.12 g $MgSO_4$, 42.8 ml propylene glycol, 11.5 ml ethyl alcohol and water to volume. Initial dose was 3.5 ml/kg and supplemental doses of up to 1 ml/kg, as needed.

conventional manner with a single platinum wire and using a window discriminator to separate spikes in multifiber preparations. The output of the window discriminator triggered a spike generator to produce a pulse which was recorded on a pen recorder along with temperature.

After isolation the afferent was studied for 40 to 60 minutes by alternating the temperature of the fluid bathing the metal thermode about every 4 minutes between cold (22 to 24°C) and warm (37 to 38°C) temperatures. In most of the multifiber preparations the mechanoreceptors all had their receptive fields in the same general skin area. We have assumed that a similar general relationship holds for receptive fields of cold and mechanoreceptors in order to direct the ouabain application to the receptive field of nonmechanically activated afferents. For each fiber the effect of local injection was tested by subcutaneous administration of mammalian Ringer's solution into the receptor region. If this did not change the discharge pattern, a 10^{-3} to 10^{-5} M solution of ouabain pH 7.4 was injected and temperature sensitivity followed with time.

III. RESULTS AND DISCUSSION

A total of 36 fibers were examined. Because of the uncertainty that the ouabain reached the receptor terminals, this report considers only those fibers which showed a response to ouabain (5 cold fibers and 11 temperature sensitive mechanoreceptors). Ouabain caused a dramatic increase in the static discharge frequencies of both cold and mechanosensitive fibers at near body temperature but usually did not change discharge by any significant amount at lower temperatures. Figure 1 illustrates the effect of ouabain on the static discharge frequency of a typical temperature

sensitive mechanoreceptor. The fiber was silent at near body temperature but had a slow discharge at 22°C. The control injection of saline had no influence on the discharge rate at either temperature. After ouabain this receptor showed a high static discharge rate at 38°C with a peak of 11 impulses per second at about 8 minutes after the ouabain infiltration, whereas the static discharge rate at 22°C remained relatively unchanged.

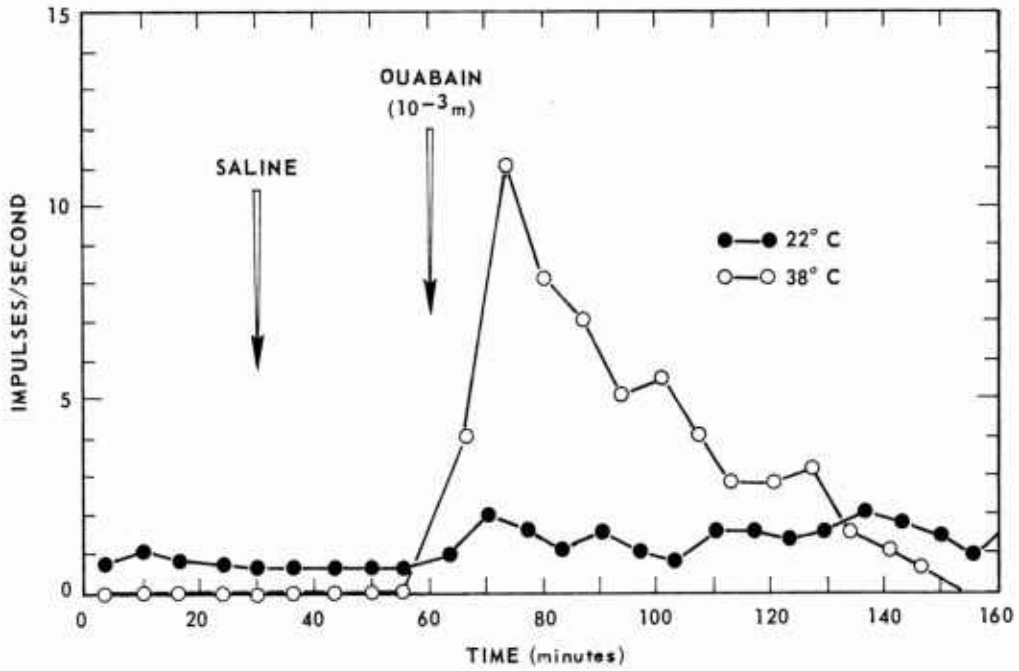


Figure 1. Effect of ouabain infiltration on the static discharge of a single cold-sensitive mechanoreceptor of rat scrotal skin at 22°C and 38°C. Each point represents the average impulse frequency between 150-180 seconds after a change in temperature. Arrows indicate times of local infiltration of 0.5 ml saline and ouabain.

Figure 2 shows actual records of a similar experiment on a cold fiber which could not be excited by mechanical stimuli and is therefore presumed to be a specific thermoreceptor. This fiber also was silent at body temperature but discharged at a

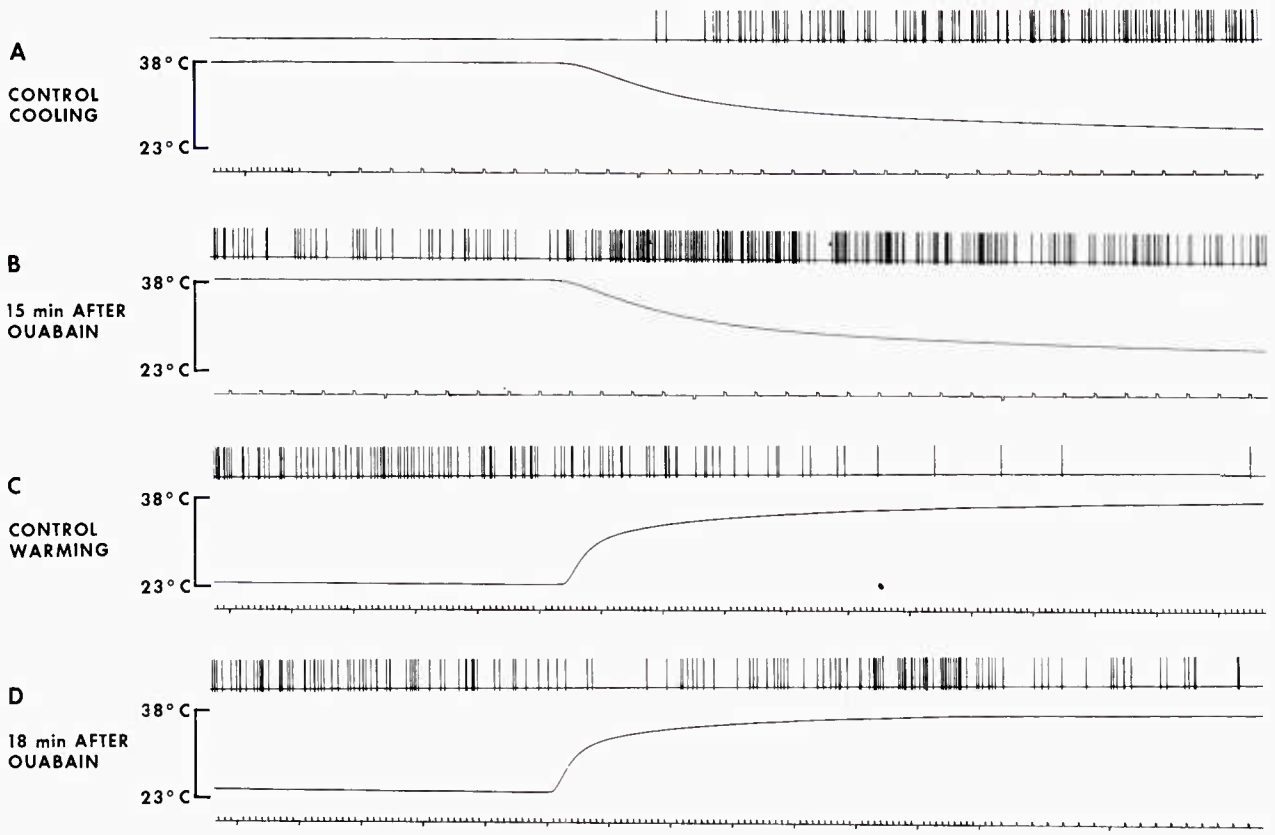


Figure 2. Effect of ouabain infiltration on static and dynamic temperature responses of a cold afferent fiber from rat scrotal skin. Upper trace: fiber discharge; middle trace: temperature recorded at the thermode-skin interface; lower trace: time marker, 1 second. Note different time scales in A and B as compared to C and D.

frequency of 1 to 2 impulses per second at 24°C. With the relatively slow temperature changes studied, there is no clear dynamic response. After ouabain the fiber discharges at body temperature at a frequency at least as great as in the cold while discharge at 24°C is relatively unchanged.

These results strongly suggest that an electrogenic sodium pump is the mechanism responsible for the temperature sensitivity of both specific cold receptors and mechanoreceptors. We propose, as has been shown for other preparations,[2, 3] that the sodium pump in these afferent terminals has an unequal coupling of active sodium

efflux and potassium influx and that the excess sodium constitutes a net outward positive current which at higher temperatures tends to hyperpolarize and decrease the excitability of the afferent terminals. The extent of this hyperpolarizing force is indicated by the change in discharge frequency after ouabain at warm temperatures. Since sodium transport is very temperature dependent, Q_{10} of at least 2.3,[1] the lack of ouabain sensitivity in the cold is best explained by a very low rate of pump activity leaving terminal excitability determined essentially by the passive membrane mechanisms.[2,3]

Application of ouabain by a local infiltration in the peripheral receptive field is clearly not a totally satisfactory technique, since one can never be sure whether any or what concentration of ouabain reaches the terminals. In a number of preparations we observed no effect of ouabain infiltration, and presume this was because of misplacement of the injection. In other preparations, as in Figure 2, the pump inhibition was probably incomplete since the discharge in the warm after ouabain never exceeded that in the cold, and transient acceleration on cooling remained. As a result it is not possible to interpret absolute dynamic or static frequencies except to note the changes after ouabain and on recovery from ouabain. However, in those fibers which were affected by ouabain the effects were reproducible on multiple application and were consistent with the observations made previously on Aplysia neurons.

It is apparent that an electrogenic sodium pump is not the only temperature-dependent process imparting thermosensitivity to these afferent fibers since, as is shown in Figure 1, the receptor after ouabain changes from being cold sensitive to

being warm sensitive. Nevertheless these experiments do support the hypothesis that the primary generator potential mechanism causing a specific thermosensitive afferent to respond to cold is the operation of an electrogenic pump which causes the terminals to be depolarized on cooling and hyperpolarized on warming.

REFERENCES

1. Albers, R. W. Biochemical aspects of active transport. Ann. Rev. Biochem. 36: 727-756, 1967.

2. Carpenter, D. O. Membrane potential produced directly by the Na^+ pump in Aplysia neurons. Comp. Biochem. Physiol. 35:371-385, 1970.

3. Carpenter, D. O. and Alving, B. O. A contribution of an electrogenic Na^+ pump to membrane potential in Aplysia neurons. J. Gen. Physiol. 52:1-21, 1968.

4. Hensel, H. and Boman, K. K. A. Afferent impulses in cutaneous sensory nerves in human subjects. J. Neurophysiol. 23:564-578, 1960.

5. Hensel, H. and Zotterman, Y. Quantitative Beziehungen zwischen der Entladung einzelner Kältefasern und der Temperatur. Acta Physiol. Scand. 23:291-319, 1951.

6. Iggo, A. Cutaneous thermoreceptors in primates and sub-primates. J. Physiol. (London) 200:403-430, 1969.

7. Iggo, A. The mechanisms of biological temperature reception. In: Physiological and Behavioral Temperature Regulation, Hardy, J. D., Gagge, A. P. and Stolwijk, J. A. J., editors, p. 391. Springfield, Illinois, Charles C. Thomas, 1970.

8. Klee, M. R., Pierau, F.-K. and Faber, D. S. Temperature effects on resting potential and spike parameters of cat motoneurons. Exptl. Brain Res. 19:478-492, 1974.

9. Lippold, O. C. J., Nicholls, J. G. and Redfearn, J. W. T. A study of the afferent discharge produced by cooling a mammalian muscle spindle. J. Physiol. (London) 153:218-231, 1960.

10. Loewenstein, W. R. On the "specificity" of a sensory receptor. J. Neurophysiol. 24:150-158, 1961.

UNCLASSIFIED
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Armed Forces Radiobiology Research Institute Defense Nuclear Agency Bethesda, Maryland 20014	UNCLASSIFIED
	2b. GROUP N/A

3. REPORT TITLE

MAMMALIAN COLD RECEPTOR AFFERENTS: ROLE OF AN ELECTROGENIC SODIUM PUMP IN SENSORY TRANSDUCTION

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)

D. O. Carpenter, Fr.-K. Pierau and P. J. Torrey

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
July 1974	10	10

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. NWED QAXM	AFRRI SR74-15
c. Task and Subtask C 912 d. Work Unit 15	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Director Defense Nuclear Agency Washington, D. C. 20305

13. ABSTRACT

The responses of specific cold-sensitive afferents and cold-sensitive mechanoreceptors were recorded from rat pudendal nerve. A local infiltration of ouabain into the region of the afferent terminals caused a dramatic increase in discharge at warm temperatures without significant effect on discharge in the cold. These results suggest that an electrogenic sodium pump is the generator potential mechanism imparting cold sensitivity.

DD FORM 1 NOV 65 1473

UNCLASSIFIED
Security Classification